1438263 PMID: 9263123

Predicting differential antigen-antibody contact regions based on solvent accessibility.

Lebeda F J; Olson M A

Department of Cell Biology and Biochemistry, U.S. Army Medical Research Institute of Infectious Diseases, Frederick, Maryland 21702-5011, USA.

Journal of protein chemistry (UNITED STATES) Aug 1997, 16 (6) p607-18, ISSN 0277-8033--Print Journal Code: 8217321

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Document type: Journal Article

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Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS; Toxbib

A novel computational approach was examined for predicting **epitopes** from primary structures of the seven immunologically distinct botulinum neurotoxins (BoNT/A-G) and tetanus toxin (TeTX). An artificial neural network [Rost and Sander (1994), Proteins 20, 216] was used to estimate residue solvent accessibilities in multiple aligned sequences. A similar network trained to predict secondary structures was also used to examine this protein family, whose tertiary fold is presently unknown. The algorithm was validated by showing that it was 80% accurate in determining the secondary structure of avian egg-white lysozyme and that it correctly identified highly solvent-exposed residues that correspond to the major contact regions of lysozyme-antibody cocrystals. When sequences of the **heavy** (H) chains of TeTX and BoNT/A-G were analyzed, this algorithm predicted that the most highly exposed regions were clustered at the sequentially nonconserved N- and C-termini [Lebeda and Olson (1994), Proteins 20, 293]. The secondary structures and the remaining highly solvent-accessible regions were, in contrast, predicted to be conserved. In experiments reported by others, H-chain **fragments**0 that induced immunological protection against BoNT/A **overlap** with these predicted most highly exposed regions. It is also known that the C-terminal halves of the TeTX and BoNT/A H-chains interfere with holotoxin binding to ectoacceptors on nerve endings. Thus, the present results provide a theoretical framework for predicting the sites that could assist in the development of genetically engineered vaccines and that could interact with neurally located toxin ectoacceptors. Finally, because the most highly solvent-exposed regions were not well conserved, it is hypothesized that nonconserved, potential contact sites partially account for the existence of different dominant binding regions for type-specific neutralizing antibodies.

Descriptors: *Algorithms; *Antigen-Antibody Reactions; *Botulinum Toxins --chemistry--CH; * **Epitopes** --chemistry--CH; *Tetanus Toxin--chemistry--CH ; **Botulinum Toxins** --immunology--IM; Neural Networks (Computer); Protein Structure, Secondary; Solvents--chemistry--CH; Tetanus Toxin--immunology --IM

to 3.9-fold compared to the control. The results suggest that there are two "productive" receptor binding sites on H(C) which lead to toxin internalization and toxicity. Blockade of these two **epitopes** with monoclonal antibodies may provide effective immunoprophylaxis or therapy against BoNT/A intoxication.

Tags: Male

Descriptors: *Antibodies, Bacterial--immunology--IM; * **Botulinum Toxin Type A** --immunology--IM; Amino Acid Sequence; Animals; Antibodies, Bacterial--genetics--GE; Antibody Affinity; Antibody Specificity; Binding Sites; Botulinum Toxin Type A--metabolism--ME; Coliphages--genetics--GE; **Epitope** Mapping; Immunoglobulin **Fragments** --immunology--IM; Kinetics; Mice; Molecular Sequence Data; **Peptide** Library; Research Support, U.S. Gov't, Non-P.H.S.

Molecular Sequence Databank No.: GENBANK/AF003702; GENBANK/AF003703; GENBANK/AF003704; GENBANK/AF003705; GENBANK/AF003706; GENBANK/AF003707; GENBANK/AF003708; GENBANK/AF003709; GENBANK/AF003710; GENBANK/AF003711; GENBANK/AF003712; GENBANK/AF003713; GENBANK/AF003714; GENBANK/AF003715; GENBANK/AF003716; GENBANK/AF003717; GENBANK/AF003718; GENBANK/AF003719; GENBANK/AF003720; GENBANK/AF003721; GENBANK/AF003722; GENBANK/AF003723; GENBANK/AF003724; GENBANK/AF003725

CAS Registry No.: 0 (Antibodies, Bacterial); 0 (Botulinum Toxin Type A); 0 (Immunoglobulin Fragments); 0 (Peptide Library)

Record Date Created: 19970919

Record Date Completed: 19970919

33/9/7 (Item 7 from file: 155)

11438263 PMID: 9263123

Predicting differential antigen-antibody contact regions based on solvent accessibility.

Lebeda F J; Olson M A

Department of Cell Biology and Biochemistry, U.S. Army Medical Research Institute of Infectious Diseases, Frederick, Maryland 21702-5011, USA.

Journal of protein chemistry (UNITED STATES) Aug 1997, 16 (6) p607-18, ISSN 0277-8033--Print Journal Code: 8217321

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS; Toxbib

A novel computational approach was examined for predicting **epitopes** from primary structures of the seven immunologically distinct botulinum neurotoxins (BoNT/A-G) and tetanus toxin (TeTX). An artificial neural network [Rost and Sander (1994), Proteins 20, 216] was used to estimate residue solvent accessibilities in multiple aligned sequences. A similar network trained to predict secondary structures was also used to examine this protein family, whose tertiary fold is presently unknown. The algorithm was validated by showing that it was 80% accurate in determining the secondary structure of avian egg-white lysozyme and that it correctly identified highly solvent-exposed residues that correspond to the major contact regions of lysozyme-antibody cocrystals. When sequences of the **heavy** (H) chains of TeTX and BoNT/A-G were analyzed, this algorithm predicted that the most highly exposed regions were clustered at the sequentially nonconserved N- and C-termini [Lebeda and Olson (1994), Proteins 20, 293]. The secondary structures and the remaining highly solvent-accessible regions were, in contrast, predicted to be conserved. In

33/9/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12721492 PMID: 10820725
Phage libraries for generation of anti-botulinum scFv antibodies.
Amersdorfer P; Marks J D
Phylos Inc., Lexington, MA, USA.
Methods in molecular biology (Clifton, N.J.) (UNITED STATES) 2000, 145 p219-40, ISSN 1064-3745--Print Journal Code: 9214969
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS; Toxbib
Descriptors: *Antibodies, Monoclonal--biosynthesis--BI; *Bacteriophages --genetics--GE; * **Botulinum Toxins** --immunology--IM; *Immunoglobulin **Fragments** --biosynthesis--BI; * **Peptide**0 Library; Animals; Antibodies, Monoclonal--immunology--IM; Bacteriophages--immunology--IM; Genes, Immunoglobulin; Immunoglobulin **Fragments** --genetics--GE; Immunoglobulin **Fragments** --immunology--IM; Immunoglobulin **Heavy** Chains--genetics--GE; Immunoglobulin **Heavy** Chains--immunology--IM; Immunoglobulin Light Chains --genetics--GE; Immunoglobulin Light Chains--immunology--IM; Immunoglobulin Variable Region--genetics--GE; Immunoglobulin Variable Region--immunology--IM; Mice; Polymerase Chain Reaction--methods--MT; Spleen--immunology--IM
CAS Registry No.: 0 (Antibodies, Monoclonal); 0 (Botulinum Toxins); 0 (Immunoglobulin Fragments); 0 (Immunoglobulin Heavy Chains); 0 (Immunoglobulin Light Chains); 0 (Immunoglobulin Variable Region); 0 (Peptide Library)
Record Date Created: 20000711
Record Date Completed: 20000711

33/9/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12478661 PMID: 10422600
Structure, activity, and immune (T and B cell) recognition of botulinum neurotoxins.
Atassi M Z; Oshima M
Department of Biochemistry, Baylor College of Medicine, Houston, Texas 77030, USA.
Critical reviews in immunology (UNITED STATES) 1999, 19 (3) p219-60 , ISSN 1040-8401--Print Journal Code: 8914819
Publishing Model Print
Document type: Journal Article; Review
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS; Toxbib
Botulism, which was first reported over a century ago, is caused by botulinum neurotoxins produced by Clostridium botulinum in seven immunological serotypes (A through G). The primary structures of a number of these BoNTs have been determined and are reviewed here, together with their gene structure and synthesis. The biological actions of BoNTs, which

result in their ability to block neurotransmitter release have been the subject of intensive study, and in this review we discuss the binding of BoNTs to the cell surface as well as the mechanism of their intercellular action. The ability of BoNTs to block neurotransmitter release has been exploited in therapeutic applications to reduce muscle hyperactivity for the treatment of a variety of clinical conditions associated with involuntary muscle spasm and contractions. The advantages, limitations, and risks of these applications are discussed. Certain compounds provide some limited protection against BoNT. However, more effective protection has been obtained immunologically either by passive immunity (i.e., by administration of anti-BoNT Abs) or by immunization with inactivated toxin. More recently, excellent protection has been obtained by immunization with the receptor-binding region comprising the C-terminal (residues 860 to 1296) **fragment** (Hc) of the **heavy** chain of BoNT/A. Here we review the mapping of the **epitopes** on the Hc region of BoNT/A that are recognized by anti-BoNT/A Abs raised in horse, human, and mouse. The **epitopes** on the Hc that are recognized by anti-Hc Abs and by Hc-primed T lymphocytes were mapped in two mouse strains [BALB/c (H-2d) and SJL (H-2s)]. The **peptides** , which contain Ab or T cell **epitopes** (or both) on the Hc, were used as immunogens in BALB/c and SJL mice and we identified those **peptides** whose Ab and/or T-cell response cross-react with Hc. Identification of these **peptides** is an important first step in the intricate requirements for the design of a synthetic vaccine. (27 Refs.)

Descriptors: *B-Lymphocytes--immunology--IM; * **Botulinum Toxins** --immunology--IM; *T-Lymphocytes--immunology--IM; Amino Acid Sequence; Animals; Botulinum Toxins--chemistry--CH; Botulinum Toxins--poisoning--PO; Humans; Immunity, Cellular; Molecular Sequence Data; Poisoning --drug therapy--DT; Sequence Homology, Amino Acid

CAS Registry No.: 0 (Botulinum Toxins)

Record Date Created: 19990831

Record Date Completed: 19990831

33/9/5 (Item 5 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

11546491 PMID: 9388028

Identification and characterization of a neutralizing monoclonal antibody against botulinum neurotoxin serotype F, following vaccination with active toxin.

Brown D R; Lloyd J P; Schmidt J J

Department of Immunology and Molecular Biology, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21702-5011, USA.

Hybridoma (UNITED STATES) Oct 1997, 16 (5) p447-56, ISSN 0272-457X --Print Journal Code: 8202424

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS; Toxbib

Clostridium botulinum may produce any of seven known serotypes of neurotoxin (BoNT/A-/G), which are the most toxic bacterial proteins known. Efforts to develop a second-generation vaccine to these toxins would benefit from the isolation of hybridomas producing neutralizing monoclonal antibodies (MAbs). We hypothesized that previous efforts to isolate neutralizing MAbs against various BoNTs failed due to use of toxoided, chemically altered antigens. We employed a novel vaccination regimen

employing native, active, single-chain BoNT/E (scBoNT/E). A number of the BoNT/E immunized mice were further vaccinated with lethal doses of fully active BoNT/F. MAb 7F8 consistently neutralized BoNT/F in three different assays: in vivo neutralization, passive neutralization, and neutralization of regional paralysis. There was no detectable recognition and essentially no neutralization of scBoNT/E. The **epitope** recognized by this MAb was denatured when treated with formalin, urea, guanidine chloride, or sodium dodecyl sulfate. Preliminary **epitope** mapping studies indicate that the MAb bound to a conformational **epitope**.

Descriptors: *Antibodies, Monoclonal--chemistry--CH; * **Botulinum Toxins** --immunology--IM; Animals; Antibodies, Monoclonal--immunology--IM; **Epitope** Mapping; Formaldehyde--pharmacology--PD; Hybridomas--metabolism--ME; Immunoglobulin **Heavy** Chains--chemistry--CH; Immunoglobulin **Heavy** Chains--isolation and purification--IP; Immunoglobulin Light Chains --chemistry--CH; Immunoglobulin Light Chains--isolation and purification --IP; Mice; Mice, Inbred BALB C; Protein Denaturation; Vaccination

CAS Registry No.: 0 (Antibodies, Monoclonal); 0 (Botulinum Toxins); 0 (Immunoglobulin Heavy Chains); 0 (Immunoglobulin Light Chains); 0 (botulinum toxin type F); 50-00-0 (Formaldehyde)

Record Date Created: 19980127

Record Date Completed: 19980127

33/9/6 (Item 6 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

11455792 PMID: 9284147

Molecular characterization of murine humoral immune response to botulinum neurotoxin type A binding domain as assessed by using phage antibody libraries.

Amersdorfer P; Wong C; Chen S; Smith T; Deshpande S; Sheridan R; Finnern R; Marks J D

Department of Anesthesia and Pharmaceutical Chemistry, University of California, San Francisco, 94110, USA.

Infection and immunity (UNITED STATES) Sep 1997, 65 (9) p3743-52, ISSN 0019-9567--Print Journal Code: 0246127

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS; Toxbib

To produce antibodies capable of neutralizing botulinum neurotoxin type A (BoNT/A), the murine humoral immune response to BoNT/A binding domain (H(C)) was characterized at the molecular level by using phage antibody libraries. Mice were immunized with BoNT/A H(C), the spleens were harvested, and single-chain Fv (scFv) phage antibody libraries were constructed from the immunoglobulin **heavy** and light chain variable region genes. Phage expressing BoNT/A binding scFv were isolated by selection on immobilized BoNT/A and BoNT/A H(C). Twenty-eight unique BoNT/A H(C) binding scFv were identified by enzyme-linked immunosorbent assay and DNA sequencing. **Epitope** mapping using surface plasmon resonance in a BIAcore revealed that the 28 scFv bound to only 4 nonoverlapping **epitopes** with equilibrium constants (Kd) ranging from 7.3 x 10(-8) to 1.1 x 10(-9) M. In a mouse hemidiaphragm assay, scFv binding **epitopes** 1 and 2 significantly prolonged the time to neuroparalysis, 1.5- and 2.7-fold, respectively, compared to toxin control. scFv binding to **epitopes** 3 and 4 showed no protection against neuroparalysis. A combination of scFv binding **epitopes** 1 and 2 had an additive effect on time to neuroparalysis, which increased

experiments reported by others, H-chain **fragments**0 that induced immunological protection against BoNT/A **overlap** with these predicted most highly exposed regions. It is also known that the C-terminal halves of the TeTX and BoNT/A H-chains interfere with holotoxin binding to ectoacceptors on nerve endings. Thus, the present results provide a theoretical framework for predicting the sites that could assist in the development of genetically engineered vaccines and that could interact with neurally located toxin ectoacceptors. Finally, because the most highly solvent-exposed regions were not well conserved, it is hypothesized that nonconserved, potential contact sites partially account for the existence of different dominant binding regions for type-specific neutralizing antibodies.

Descriptors: *Algorithms; *Antigen-Antibody Reactions; *Botulinum Toxins --chemistry--CH; * **Epitopes** --chemistry--CH; *Tetanus Toxin--chemistry--CH ; **Botulinum Toxins** --immunology--IM; Neural Networks (Computer); Protein Structure, Secondary; Solvents--chemistry--CH; Tetanus Toxin--immunology --IM

CAS Registry No.: 0 (Botulinum Toxins); 0 (Epitopes); 0 (Solvents); 0 (Tetanus Toxin)

Record Date Created: 19970925

Record Date Completed: 19970925

33/9/8 (Item 8 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

11420606 PMID: 9246568

Localization of the regions on the C-terminal domain of the heavy **chain of botulinum A recognized by T lymphocytes and by antibodies after immunization of mice with pentavalent toxoid.**

Rosenberg J S; Middlebrook J L; Atassi M Z

Department of Biochemistry, Baylor College of Medicine, Houston, TX 77030, USA.

Immunological investigations (UNITED STATES) Jun 1997, 26 (4) p491-504, ISSN 0882-0139--Print Journal Code: 8504629

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS; Toxbib

We have mapped the regions recognized by T and/or B cells (Abs) on the C-terminal domain (Hc) of the **heavy** chain of botulinum neurotoxin serotype A (BoNT/A) after immunization of two inbred mouse strains with pentavalent toxoid (BoNTs A, B, C, D and E). Using a set of synthetic **overlapping peptides** , encompassing the entire Hc domain (residues 855-1296), we demonstrated that T cells of Balb/c (H-2d) mice, primed with one injection of toxoid, recognized two major regions within residues 897-915 and 939-957. After multiple inoculations with toxoid, T cells of Balb/c expanded their recognition ability and responded very well to challenge with **peptide** 1261-1279 and moderately to stimulation with **peptide** 1149-1167. Unlike Balb/c T cells, those of toxoid-primed SJL (H-2s) mice exhibited a more complex profile and responded to challenge with a large number of **overlapping peptides** . After one toxoid injection, however, three **peptides** , 897-915, 939-957/953-971 **overlap**0 and 1051-1069, were the most potent T cells stimulators. After three toxoid injections, **peptides** 897-915 and 1051-1069 remained immunodominant while the third region was shifted upstream to 925-943/939-957 **overlap** . The immunodominant **epitope** within **peptide** 897-915 was recognized

exclusively by T cells, since no Abs were detected against this region. The Ab binding profiles of the two mouse strains were quite similar, showing only small quantitative differences. Both, Balb/c and SJL anti-toxoid Abs displayed strong binding mainly to **peptide** 1177-1195, followed by **peptides** 869-887/883-901 **overlap** and 1275-1296. In addition, a significant amount of Balb/c anti-toxoid Abs was bound to **peptide** 1135-1153. Unlike Balb/c Abs, that interacted weakly with **peptides** 995-1013 and 1051-1069, the anti-toxoid Abs of SJL mice exhibited strong binding toward both **peptides** . The results showed that, in a given strain, the regions recognized by anti-toxoid Abs and T cells may coincide or may be uniquely B or T cell determinants.

Tags: Female

Descriptors: *Antibodies, Bacterial; *Botulinum Toxin Type A--chemistry --CH; * **Botulinum Toxin Type A** --immunology--IM; *T-Lymphocytes --immunology--IM; Amino Acid Sequence; Animals; Bacterial Vaccines --isolation and purification--IP; Botulinum Toxin Type A--genetics--GE; Clostridium botulinum--immunology--IM; **Epitope** Mapping; Immunization; Lymphocyte Activation; Mice; Mice, Inbred BALB C; Molecular Sequence Data; **Peptide Fragments** --chemistry--CH; **Peptide Fragments** --genetics--GE; **Peptide Fragments** --immunology--IM; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, Non-P.H.S.; Toxoids --administration and dosage--AD

CAS Registry No.: 0 (Antibodies, Bacterial); 0 (Bacterial Vaccines); 0 (Botulinum Toxin Type A); 0 (Clostridium botulinum toxoid); 0 (Peptide Fragments); 0 (Toxoids)

Record Date Created: 19970929

Record Date Completed: 19970929

33/9/9 (Item 9 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

11312752 PMID: 9125539

Antibody mapping to domains of botulinum neurotoxin serotype A in the complexed and uncomplexed forms.

Chen F; Kuziemko G M; Amersdorfer P; Wong C; Marks J D; Stevens R C

Graduate Group in Biophysics, University of California, Berkeley 94720, USA.

Infection and immunity (UNITED STATES) May 1997, 65 (5) p1626-30, ISSN 0019-9567--Print Journal Code: 0246127

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS; Toxbib

The domain organization of the botulinum neurotoxin serotype A was studied by using antibody mapping of 44 monoclonal single-chain variable **fragments** . The analysis was carried out on (i) the individual domains of botulinum neurotoxin holotoxin (binding, **translocation** , and catalytic), (ii) botulinum neurotoxin holotoxin, (iii) the botulinum neurotoxin holotoxin in complex with the nontoxic portion, and (iv) botulinum neurotoxin holotoxin and nontoxic portion of the complex recombined in vitro. All 44 antibodies mapped to individual domains of botulinum neurotoxin. Forty of the 44 single-chain variable **fragments** bound the botulinum neurotoxin holotoxin relative to the isolated domains, suggesting that 4 **epitopes** are covered when the individual domains are in the holotoxin form. Only 20 of the antibodies showed a positive reaction to the toxin while in complex with the nontoxic portion. All of the covered

Monoclonal); 0 (Botulinum Toxins); 0 (Epitopes); 0 (botulinum toxin type E)
Record Date Created: 19970709
Record Date Completed: 19970709

33/9/11 (Item 11 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11169189 PMID: 9014296
Mapping of protective and cross-reactive domains of the type A neurotoxin of Clostridium botulinum.
Dertzbaugh M T; West M W
Toxinology Division, U.S. Army Medical Research Institute of Infectious Diseases, Frederick, MD 21702-5011, USA.
Vaccine (ENGLAND) Nov 1996, 14 (16) p1538-44, ISSN 0264-410X--Print
Journal Code: 8406899
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS; Toxbib
The purpose of this study was to identify the location of domains within the serotype A neurotoxin of Clostridium botulinum (BoNT/A) that conferred protection against botulism. The BoNT/A gene was subcloned into a series of 10 **overlapping fragments** that were expressed in Escherichia coli. The expressed proteins were partially purified and used to immunize mice. The resulting antisera were screened by immunoblotting analysis for the presence of BoNT/A-specific antibody. All **fragments** , except one, elicited antibody that recognized BoNT/A in an immunoblot. Serological screening identified several **fragment** -specific cross-reactive **epitopes** that were shared by heterologous serotypes of BoNT. Most of these **epitopes** immunoreactive by enzyme-linked immunosorbent assay, but not by immunoblot. Only two **fragments** were shown to confer protection against BoNT/A intoxication. Both of these proteins were derived from segments of the **heavy** chain and encoded amino acid residues H455-661 and H1150-1289 of BoNT/A.
Tags: Female
Descriptors: ***Botulinum Toxin Type A** --immunology--IM; *Clostridium botulinum--immunology--IM; * **Epitope** Mapping--methods--MT; Animals; Botulinum Toxin Type A--biosynthesis--BI; Botulinum Toxin Type A --isolation and purification--IP; Botulism--prevention and control--PC; Cross Reactions; Enzyme-Linked Immunosorbent Assay; Immunoblotting; Mice; Mice, Inbred C57BL; Protein Structure, Tertiary
CAS Registry No.: 0 (Botulinum Toxin Type A)
Record Date Created: 19970401
Record Date Completed: 19970401

33/9/12 (Item 12 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10001088 PMID: 8132537
Specific antibodies against the Zn(2+)-binding domain of clostridial neurotoxins restore exocytosis in chromaffin cells treated with tetanus or botulinum A neurotoxin.
Bartels F; Bergel H; Bigalke H; Frevert J; Halpern J; Middlebrook J

Institute of Toxicology, Medical School of Hannover, Germany.
Journal of biological chemistry (UNITED STATES) Mar 18 1994, 269 (11) p8122-7, ISSN 0021-9258--Print Journal Code: 2985121R
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS; Toxbib

Although tetanus and botulinum A neurotoxins are ineffective in cultured chromaffin cells, they will inhibit carbachol-induced release of noradrenaline provided they gain access to the cytosol either through artificial **pores** generated in the plasma membrane or by binding to incorporated exogenous gangliosides. The block of exocytosis persists for weeks followed by a slow recovery of cell function. When specific anti-botulinum A toxin antibodies are introduced into cells through **pores** after manifestation of the block by botulinum A neurotoxin, restoration of exocytotic function is accelerated and fully reestablished within 4 days. The same time course of restoration is seen with anti-tetanus toxin antibodies in cells poisoned by tetanus toxin. Since the light chains of the toxins are enzymatically active, we have introduced polyclonal and monoclonal anti-light chain antibodies into the cytosol. Of all light chain antibodies tested, only those directed against the **peptide** homologous to the zinc-binding sequence, which is present in both neurotoxins, restored exocytosis regardless of which toxin caused the block. These results indicate that the zinc-binding domain is directly involved in the interaction of the light chains with their substrates and that the toxins have to be present continuously to maintain the block.

Descriptors: *Adrenal Medulla--metabolism--ME; *Antibodies--pharmacology --PD; *Antibodies, Monoclonal--pharmacology--PD; *Botulinum Toxins --toxicity--TO; *Exocytosis--drug effects--DE; *Gangliosides--metabolism --ME; *Norepinephrine--metabolism--ME; *Tetanus Toxin--toxicity--TO; *Zinc --metabolism--ME; Adrenal Medulla--drug effects--DE; Amino Acid Sequence; Animals; **Botulinum Toxins** --immunology--IM; Carbohydrate Sequence; Cattle; Cells, Cultured; Enzyme-Linked Immunosorbent Assay; Kinetics; Macromolecular Substances; Molecular Sequence Data; Neurotoxins--immunology --IM; Neurotoxins--toxicity--TO; Research Support, Non-U.S. Gov't; Sequence Homology, Amino Acid; Tetanus Toxin--immunology--IM

CAS Registry No.: 0 (Antibodies); 0 (Antibodies, Monoclonal); 0 (Botulinum Toxins); 0 (Gangliosides); 0 (Macromolecular Substances); 0 (Neurotoxins); 0 (Tetanus Toxin); 51-41-2 (Norepinephrine); 7440-66-6 (Zinc)

Record Date Created: 19940418
Record Date Completed: 19940418

33/9/37 (Item 18 from file: 73)
DIALOG(R)File 73:EMBASE

07843031 EMBASE No: 1999090499
Generation of a histidine-tagged antibotulinum toxin antibody fragment **in E. coli: Effects of post-induction temperature on yield and IMAC binding-affinity**

Bentley W.E.; Madurawe R.D.; Gill R.T.; Shiloach M.; Chase T.E.; Pulliam-Holoman T.R.; Valdes J.J.
Dr. W.E. Bentley, Department of Chemical Engineering, Center Agricultural Biotechnology, University of Maryland, College Park, MD 20742 United States
Journal of Industrial Microbiology and Biotechnology (J. IND. MICROBIOL. BIOTECHNOL.) (United Kingdom) 1998, 21/6 (275-282)

CODEN: JIMBF ISSN: 1367-5435
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 23

Recombinant E. coli clones expressing a 50-kDa poly-histidine tail tagged antibody **fragment** against botulinum toxin (bt-Fab) were initially screened for yield and binding affinity. One clone was selected for bioprocess development. The selected bt-Fab vector was induced by addition of IPTG and the protein was targeted to the periplasm by inclusion of a pelB leader sequence. A histidineinf 6 affinity ligand at the **heavy** chain C-terminus facilitated single-step purification by immobilized metal-affinity chromatography (IMAC). Notably, the effects of post-induction temperature on bt-Fab expression and downstream purification were evaluated. Our results demonstrated that fermentation conditions interfered with purification on the IMAC column at 37degreeC. Protease analysis by gelatin polyacrylamide gel electrophoresis (GPAGE) indicated the presence of a membrane-bound ~ 39 kDa protease activity shortly after induction. The appearance of the protease activity was inversely correlated with the bt-Fab yield. The protease was purified and was shown to degrade bt-Fab. A simple kinetic model was developed describing temporal regulation of protease and bt-Fab degradation. Partially degraded bt-Fab was unrecoverable by IMAC, presumably due to the loss of the Hisinf 6 affinity ligand. The amount of purified bt-Fab obtained per liter of fermentation broth was typically ~ 1 mg.

DRUG DESCRIPTORS:
*histidine; * **botulinum antiserum** ; *immunoglobulin f(ab) **fragment** ; * recombinant antibody
botulinum toxin; isopropyl thiogalactoside; signal **peptide** ; proteinase
MEDICAL DESCRIPTORS:
escherichia coli; temperature sensitivity; binding affinity; protein targeting; affinity chromatography; protein purification; polyacrylamide gel electrophoresis; enzyme activity; protein degradation; kinetics; nonhuman; article
CAS REGISTRY NO.: 645-35-2, 7006-35-1, 71-00-1 (histidine); 26112-89-0 (isopropyl thiogalactoside); 9001-92-7 (proteinase)
SECTION HEADINGS:
004 Microbiology: Bacteriology, Mycology, Parasitology and Virology
026 Immunology, Serology and Transplantation
052 Toxicology

33/9/53 (Item 34 from file: 73)
DIALOG(R)File 73:EMBASE

05792305 EMBASE No: 1994200996
Specific antibodies against the Znsup 2sup +-binding domain of clostridial neurotoxins restore exocytosis in chromaffin cells treated with tetanus or botulinum A neurotoxin
Bartels F.; Bergel H.; Bigalke H.; Frevert J.; Halpern J.; Middlebrook J.
Medical School of Hannover,30625 Hannover Germany
Journal of Biological Chemistry (J. BIOL. CHEM.) (United States) 1994 , 269/11 (8122-8127)
CODEN: JBCHA ISSN: 0021-9258
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LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

Although tetanus and botulinum A neurotoxins are ineffective in cultured chromaffin cells, they will inhibit carbachol-induced release of

noradrenaline provided they gain access to the cytosol either through artificial **pores** generated in the plasma membrane or by binding to incorporated exogenous gangliosides. The block of exocytosis persists for weeks followed by a slow recovery of cell function. When specific anti-botulinum A toxin antibodies are introduced into cells through **pores** after manifestation of the block by botulinum A neurotoxin, restoration of exocytotic function is accelerated and fully reestablished within 4 days. The same time course of restoration is seen with anti-tetanus toxin antibodies in cells poisoned by tetanus toxin. Since the light chains of the toxins are enzymatically active, we have introduced polyclonal and monoclonal anti- light chain antibodies into the cytosol. Of all light chain antibodies tested, only those directed against the **peptide** homologous to the zinc- binding sequence, which is present in both neurotoxins, restored exocytosis regardless of which toxin caused the block. These results indicate that the zinc-binding domain is directly involved in the interaction of the light chains with their substrates and that the toxins have to be present continuously to maintain the block.

DRUG DESCRIPTORS:
* **botulinum antiserum** ; *botulinum toxin a--drug toxicity--to; *clostridium toxin--drug toxicity--to; *tetanus antibody; *tetanus toxin--drug toxicity --to
immunoglobulin light chain
MEDICAL DESCRIPTORS:
*chromaffin cell; *cytotoxicity; *exocytosis; *noradrenalin release
animal cell; antibody specificity; antigen binding; article; cattle; enzyme binding; nonhuman; priority journal; sequence homology; toxin analysis; toxin structure
CAS REGISTRY NO.: 93384-43-1 (botulinum toxin a)
SECTION HEADINGS:
  029 Clinical and Experimental Biochemistry
  052 Toxicology

1169189 PMID: 9014296

Mapping of protective and cross-reactive domains of the type A neurotoxin of Clostridium botulinum.

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Vaccine (ENGLAND) Nov 1996, 14 (16) p1538-44, ISSN 0264-410X--Print Journal Code: 8406899

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Document type: Journal Article

Languages: ENGLISH

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The purpose of this study was to identify the location of domains within the serotype A neurotoxin of Clostridium botulinum (BoNT/A) that conferred protection against botulism. The BoNT/A gene was subcloned into a series of 10 **overlapping fragments** that were expressed in Escherichia coli. The expressed proteins were partially purified and used to immunize mice. The resulting antisera were screened by immunoblotting analysis for the presence of BoNT/A-specific antibody. All **fragments** , except one, elicited antibody that recognized BoNT/A in an immunoblot. Serological screening identified several **fragment** -specific cross-reactive **epitopes** that were shared by heterologous serotypes of BoNT. Most of these **epitopes** immunoreactive by enzyme-linked immunosorbent assay, but not by immunoblot. Only two **fragments** were shown to confer protection against BoNT/A intoxication. Both of these proteins were derived from segments of the **heavy** chain and encoded amino acid residues H455-661 and H1150-1289 of BoNT/A.

11296756 PMID: 9097417

Epitope **regions in the** heavy **chain of Clostridium botulinum type E neurotoxin recognized by monoclonal antibodies.**

Kubota T; Watanabe T; Yokosawa N; Tsuzuki K; Indoh T; Moriishi K; Sanda K ; Maki Y; Inoue K; Fujii N

Department of Microbiology, Sapporo Medical University, Japan.

Applied and environmental microbiology (UNITED STATES) Apr 1997, 63 (4) p1214-8, ISSN 0099-2240--Print Journal Code: 7605801

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS; Toxbib

Seventeen monoclonal antibodies (MAbs) were previously established against the **heavy** chain (Hc) of botulinum type E neurotoxin in BALB/c mice immunized with the type E toxoid. Five MAbs (LE15-5, LE34-6, EK19-7, EK21-4, and AE27-9) showed toxin-neutralizing activity in mice. Two of the five MAbs, EK19-7 and EK21-4, recognized the regions located at amino acid positions 731 to 787 and 811 to 897, respectively. One of the remaining three antibodies (LE34-6) reacted with the amino acid sequence VIKAIN, at amino acid positions 663 to 668, closed by the ion **channel** -forming domain. It is suggested that the ion **channel** -forming domain may also be associated with the blocking of acetylcholine release. Furthermore, the amino acid sequence YLTHMRD within 30 residues of the C-terminal region of the Hc component seemed to be recognized by LE15-5. It has been reported that the binding domain of the type E toxin is located on the C-terminal half of the Hc component. Therefore, the neutralizing activity of LE15-5 antibody may be attributed to its ability to block the binding of neurotoxin to the receptor of target cells.

Descriptors: ***Botulinum Toxins** --immunology--IM; *Clostridium botulinum --immunology--IM; * **Epitopes** --genetics--GE; Amino Acid Sequence; Animals; Antibodies, Bacterial--immunology--IM; Antibodies, Monoclonal--immunology --IM; Botulinum Toxins--genetics--GE; Clostridium botulinum--genetics--GE; **Epitope** Mapping; **Epitopes** --immunology--IM; Mice; Mice, Inbred BALB C; Molecular Sequence Data

CAS Registry No.: 0 (Antibodies, Bacterial); 0 (Antibodies, Monoclonal); 0 (Botulinum Toxins); 0 (Epitopes); 0 (botulinum toxin type E)

Record Date Created: 19970709

Record Date Completed: 19970709

33/9/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

13275650 PMID: 11425742

Characterization of neutralizing antibodies and identification of neutralizing epitope **mimics on the Clostridium botulinum neurotoxin type A.**

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Applied and environmental microbiology (United States) Jul 2001, 67 (7) p3201-7, ISSN 0099-2240--Print Journal Code: 7605801
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Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS; Toxbib

Clostridium botulinum neurotoxin type A (BTx-A) is known to inhibit the release of acetylcholine at the neuromuscular junctions and synapses and to cause neuroparalysis and death. In this study, we have identified two monoclonal antibodies, BT57-1 and BT150-3, which protect ICR mice against lethal doses of BTx-A challenge. The neutralizing activities for BT57-1 and BT150-3 were 10(3) and 10(4) times the 50% lethal dose, respectively. Using immunoblotting analysis, BT57-1 was recognized as a light chain and BT150-3 was recognized as a **heavy** chain of BTx-A. Also, applying the phage display method, we investigated the antibodies' neutralizing B-cell **epitopes** . These immunopositive phage clones displayed consensus motifs, Asp-Pro-Leu---for-BT57-1 and Cys-X-Asp-Cys for BT150. The synthetic **peptide** P4M (KGTFDPLQEPRT) corresponded to the phage-displayed **peptide** selected by BT57-1 and was able to bind the antibodies specifically. This **peptide** was also shown by competitive inhibition assay to be able to inhibit phage clone binding to BT57-1. Aspartic acid (D(5)) in P4M was crucial to the binding of P4M to BT57-1, since its binding activity dramatically decreased when it was changed to lysine (K(5)). Finally, immunizing mice with the selected phage clones elicited a specific humoral response against BTx-A. These results suggest that phage-displayed random- **peptide** libraries are useful in identifying the neutralizing **epitopes** of monoclonal antibodies. In the future, the identification of the neutralizing **epitopes** of BTx-A may provide important information for the identification of the BTx-A receptor and the design of a BTx-A vaccine.

Descriptors: *Antibodies, Monoclonal--immunology--IM; * **Botulinum Toxin Type A** --immunology--IM; *Clostridium botulinum--immunology--IM; * **Epitopes** , B-Lymphocyte; *Molecular Mimicry; Amino Acid Sequence; Animals; Antibodies, Bacterial--biosynthesis--BI; Antibodies, Bacterial--immunology --IM; Antibodies, Monoclonal--biosynthesis--BI; Botulinum Toxin Type A --chemistry--CH; Botulinum Toxin Type A--genetics--GE; Botulism --microbiology--MI; Botulism--prevention and control--PC; Enzyme-Linked Immunosorbent Assay; **Epitopes** , B-Lymphocyte--chemistry--CH; **Epitopes** , B-Lymphocyte--genetics--GE; **Epitopes** , B-Lymphocyte--immunology--IM; Immunization; Immunoblotting; Mice; Mice, Inbred ICR; Molecular Sequence Data; Neutralization Tests; **Peptide** Library; **Peptides** --chemical synthesis--CS; **Peptides** --immunology--IM; **Peptides** --metabolism--ME; Research Support, Non-U.S. Gov't
CAS Registry No.: 0 (Antibodies, Bacterial); 0 (Antibodies, Monoclonal); 0 (Botulinum Toxin Type A); 0 (Epitopes, B-Lymphocyte); 0 (Peptide Library); 0 (Peptides)
Record Date Created: 20010626
Record Date Completed: 20010920

epitopes were mapped to the binding domain of botulinum neurotoxin, which suggested that the binding domain is in direct contact with the nontoxic portion in the complex. Based on the antibody mapping to the different domains of the botulinum neurotoxin holotoxin and the entire complex, a model of the botulinum neurotoxin complex is proposed.

Descriptors: ***Botulinum Toxin Type A** --immunology--IM; * **Epitope** Mapping; Antibodies, Monoclonal--immunology--IM; Antigen-Antibody Reactions; Binding Sites, Antibody; Botulinum Toxin Type A--isolation and purification--IP; Chromatography; Electrophoresis, Polyacrylamide Gel; Enzyme-Linked Immunosorbent Assay; Hemagglutinins--immunology--IM; **Peptides** --immunology--IM; Recombinant Proteins--immunology--IM; Research Support, U.S. Gov't, Non-P.H.S.

CAS Registry No.: 0 (Antibodies, Monoclonal); 0 (Binding Sites, Antibody); 0 (Botulinum Toxin Type A); 0 (Hemagglutinins); 0 (Peptides); 0 (Recombinant Proteins)

Record Date Created: 19970515

Record Date Completed: 19970515

33/9/10 (Item 10 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

11296756 PMID: 9097417

Epitope **regions in the** heavy **chain of Clostridium botulinum type E neurotoxin recognized by monoclonal antibodies.**

Kubota T; Watanabe T; Yokosawa N; Tsuzuki K; Indoh T; Moriishi K; Sanda K ; Maki Y; Inoue K; Fujii N

Department of Microbiology, Sapporo Medical University, Japan.

Applied and environmental microbiology (UNITED STATES) Apr 1997, 63 (4) p1214-8, ISSN 0099-2240--Print Journal Code: 7605801

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS; Toxbib

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CAS Registry No.: 0 (Antibodies, Bacterial); 0 (Antibodies,